



SYNTHESIS CHARACTERIZATION AND BIOLOGICAL SIGNIFICANCE OF ORGANOMETALLIC COMPOUNDS OF RARE EARTHS M(III) METAL IONS WITH L- ASCORBIC ACID

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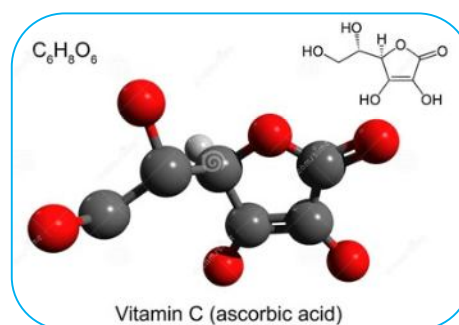
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ABSTRACT

Vitamin C (ascorbic acid) is sensitive to oxygen and heat, and can be degraded during unsuitable conditions of cooking and preservation methods of food. The nutritional quality of food may be adversely affected due to transition metal-catalyzed oxidative degradation of ascorbic acid. Complexes of La(III), Ce(III), Nd(III) and Gd(III) with Ligand L-ascorbic acid were prepared in aqueous ethanol. Complexes were characterized using UV-Visible, IR, NMR, Mass spectroscopic methods as well as magnetic susceptibility and conductivity measurements. In addition biological activity of the synthesised metal complexes against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus aureus* bacteria and *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium* and *Trichoderma viride* fungi respectively were examined in-vitro. Some of the metal complex displayed pounced biological activity.



KEYWORDS: Vitamin C, metal complex, organometallic compounds, Antimicrobial activities .

INTRODUCTION

Ascorbic acid (AA), commonly known as Vitamin C, is one of the most important water-soluble vitamins in the human diet, because it helps the body in forming connective tissue, bone, teeth, blood vessel walls, and it assists the body in assimilating iron and amino acids.[1] A diet deficient in vitamin C may cause a person to develop scurvy. Vitamin C lowers the incidence of mortality from two of the most prevalent diseases, cardiovascular disease and cancer.[2] AA is an essential vitamin for human health. It is widely found in vegetables and fruits, and is capable of quenching free radical species. It also prevents worsening of taste and colour in various foodstuffs and fruits. It can either be derived from natural sources or be chemically synthesized. Since humans cannot synthesize vitamin C in their bodies, they have to supply it from nutritional sources. All fresh vegetables, fruits, and meat contain certain amounts of vitamin C. Since this vitamin is sensitive to air oxygen and heat, it can be relatively rapidly degraded during unsuitable conditions of cooking and preservation of food. Ascorbic acid is a natural component of many foods and is often added to food and beverages as a vitamin supplement and antioxidant. AA is a good indicator of the retention of nutritional quality of fruits and vegetables,

because it is highly sensitive to temperature, humidity, and air.[6] Vitamin C in a fortified formula was shown to decrease more rapidly with increasing water activity of the medium.[3] An additional advantage of AA is its synergistic protective action on food plant flavonoids from oxidative degradation.[4] Vitamin C regenerates vitamin E by reducing vitamin E radicals formed when vitamin E scavenges the oxygen radicals. This interaction between vitamin C and vitamin E radicals can take place not only in homogeneous solutions but also in liposomal membrane systems.[5] Some evidence suggests that ascorbate protects against lipid peroxidation by regenerating the reduced form of α -tocopherol.[6] AA content is taken as an indication of fruit freshness and retention of other components, because contrary to other organic acids and sugars, vitamin C is quite unstable with respect to the activity of ascorbic acid oxidase enzyme and to the reaction with oxygen in the presence of heavy metal ions and light.[6,7] Thus, AA—due to its oxidation to de-hydro-ascorbic acid by molecular oxygen—is the most affected hydrophilic antioxidant during processing of fruits and vegetables.[7] Therefore, mechanisms governing its protection in plant food are worthy of exploration.[8-10]

Lanthanides (Rare Earth Elements—REEs) and their coordination compounds due to their luminescent properties play a crucial role in biological systems, especially in the diagnosis and monitoring of the progress of treatment of cancer diseases. Due to their luminescent properties showing a characteristic line emission after light absorption that is enhanced by the surrounding ligands [11,12]. The paramagnetic properties of lanthanide (III) ions (Ln(III)) and their complexes make them suitable for use as a contrast media for Magnetic Resonance Imaging (MRI). Particularly Gd (III) ion complexes have been found widely used in imaging diagnostics[13] Emission of lanthanide(III) ions, e.g., Nd (III), Ce (III), or La (III) in the Near-infrared (NIR) region that is detected through animal tissue of considerable thickness, could also be used for imaging in vivo [14]. Additionally, lanthanide (III) compounds are used as antibacterial agents [15–18] and show very effective catalytic properties with high selectivity for hydrolytic cleavage or Trans esterification of RNA and as a substance promoting DNA cleavage [19–21]. According to the Hard and Soft Acids and Bases (HSAB) theory, lanthanide(III) ions prefer the complexation with ligand with donor atoms in the order: O>N>S. Tartaric acid with O-donor atoms are potentially good ligands and can be assembled in a diverse arrangement as a chelating or bridging species, and coordination complexes of a mononuclear, binuclear, and polymeric type could be formed [22–25].

INSTRUMENTATION

1. Infra-red spectra between (400-4000 cm^{-1}) were performed with (FT-IR) 8300 Shimadzu spectrophotometer, The NMR spectra were recorded on a Bruker NMR spectrometer (300 MHz).
2. The electronic spectra were recorded on the UV, Visible spectrophotometer type (spectra 190-900) nm CECIL, using water as a solvent.
3. The melting point was recorded on "Gallen Kamp melting point Apparatus".
4. The conductance measurements were recorded on W.T.W. conductivity meter.
5. Elemental analysis for carbon, hydrogen was using a Euro Vector EA 3000 A Elemental Analysis .
6. Metal analysis. The metal contents of the complexes were determined by atomic absorption technique. Using a Shimadzu PR-5 ORAPHIC PRINTER atomic absorption spectrophotometer.

Synthesis of Binary Complex:

The general procedure for the synthesis of M (III) Ligand complexes: La(III), Ce(III), Nd(III), Gd(III) binary metal complexes with 5, 6-O-isopropylidene-L-ascorbic acid. The binary metal complexes were synthesized by mixing 10 ml solution of metal salts (0.01 mol) with 10 ml of L-ascorbic acid (0.01 mol) in hot ethanol by keeping the metal-Ligand ratio (1:2 v/v). The mixture was refluxed for about 4 to 6 hours on a water bath with continuous stirring. The pH of the solution was adjusting about 5 to 6 by adding an acidic buffer solution in ethanol. The volume of the solution was reduced to half. The solid coloured products thus obtained were filtered, washed with distilled water and cold ethanol and then dried in vacuum over anhydrous calcium chloride a desiccator.

Metal Complexes **3(a-d)** was synthesized in the similar manner using compound 1 and various selected Metal Chlorides. Characterization data are presented in **Table-1**

RESULT AND DISCUSSION

Metal complexes were tested for in vitro antibacterial activity against some bacterial strains using spot on lawn on Muller Hinton Agar (MHA). Four test pathogenic bacterial strains, viz., *Bacillus cereus* (MTCC 1272), where MTCC—Microbial type culture collections, *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 739), and *Staphylococcus aureus* (MTCC 1144), *Klebsiella pneumonia* (MTCC 1377) bacteria and *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxisporium* and *Trichoderma viride* fungi were considered for determination of minimum inhibitory concentration (MIC) of selected complexes. The test pathogens were sub-cultured aerobically using Brain Heart Infusion Agar (HiMedia, Mumbai, India) at 37°C (24 h). Working cultures were stored at 4°C in Brain Heart Infusion (BHI) broth (HiMedia, Mumbai, India), while stock cultures were maintained at -70°C in BHI broth containing 15% (v/v) glycerol (Qualigens, Mumbai, India). The organism was grown overnight in 10 ml of BHI broth, centrifuged at 5.000 g for 10 min, and the pellet was suspended in 10 ml of phosphate buffer saline (PBS, pH 7.2). Optical density at 545 nm (OD₅₄₅) was adjusted to obtain 10⁸ cfu/ml followed by plating serial dilution onto plate count agar (HiMedia, Mumbai, India).

Determination of MIC. MIC is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation. Antimicrobial activity of the compounds was evaluated using spot_on_lawn on MHA (HiMedia, Mumbai, India). Soft agar was prepared by adding 0.75% agar in Muller Hinton Broth (HiMedia, Mumbai, India). Soft agar was inoculated with 1% of 10⁸ Cfu/ml of the test pathogen, and 10 ml were overlaid on MHA. From 1000X solution of compound (1 mg/ml of DMSO) 1, 2, 4, 8, 16, 32, 64, and 128X solutions were prepared. Dilutions of standard antibiotics (streptomycin and griseofulvin) were also prepared in the same manner: 5 µl of the appropriate dilution was spotted on the soft agar and incubated at 37°C for 24 h. Zones of inhibition of compounds were considered after subtraction of the inhibition zone of DMSO. Negative control (with no compound) was also observed.

Antimicrobial Studies

The antibacterial tests were prepared and characterized according to the standard method. All strains were isolated from laboratory of microbiology. The identity of all the strains was confirmed. A bacterial suspension was prepared and added to the Nutrient Agar, the fungi added to surrounded Agar, All this before medium solidification and under aseptic condition. Then different concentration of complexes were placed on the surface of the culture, The bacteria incubated at 37 °C for 24 h, The fungi incubated at 28 °C for 72 h.

Bacterial & Fungi Cultures

Plate cultures of nutrient agar medium were used for culture of bacteria. the medium was prepared by dissolving 14 g, and culture of fungi's 32.5 g of powder in 500 mL of sterile distilled water, Then the medium was sterilized by autoclaving at 121 °C for 15 min.

Synthesis of Binary Complex:

The general procedure for the synthesis of M (III) Ligand complexes: La, Ce, Nd, Gd binary metal complexes with L- Ascorbic acid. The binary metal complexes were synthesized by mixing 10 ml solution of metal salts (0.01 mol) with 10 ml of L-ascorbic acid (0.01 mol) in hot ethanol by keeping the metal-Ligand ratio (1:2 v/v). The mixture was refluxed for about 4 to 6 hours on a water bath with continuous stirring. The pH of the solution was adjusting about 5 to 6 by adding an acidic buffer solution in ethanol. The volume of the solution was reduced to half. The solid coloured products thus obtained were filtered, washed with distilled water and cold ethanol and then dried in vacuum over anhydrous calcium chloride a desiccator.

Metal Complexes **3(a-d)** was synthesized in the similar manner using compound 1 and various selected Metal Chlorides. Characterization data are presented in **Table-1 to 5**

Table -1: Physical parameters of synthesised complexes $[M(AA)_2(H_2O)_2]^-$

Complexes	Colour	M.P. in °C	Elemental Analysis Calculated (Found)		
			C%	H%	M(III) %
$[La(AA)_2(H_2O)_2]^-$	White	312-314	27.53 (27.49)	3.05 (3.01)	26.56 (26.63)
$[Ce(AA)_2(H_2O)_2]^-$	Red	282-284	27.47 (27.41)	3.05 (3.01)	26.73 (26.83)
$[Nd(AA)_2(H_2O)_2]^-$	Pink	302-304	27.25 (27.19)	3.02 (2.98)	27.31 (27.38)
$[Gd(AA)_2(H_2O)_2]^-$	Brown	290-294	26.60 (26.55)	2.95 (2.92)	29.04 (29.11)

Table -2: Spectrum analysis of Synthesised metal complex 3a

Molecular Formula	$[La(AA)_2(H_2O)_2]^-$
Chemical Formula	$[C_{12}H_{14}O_{12}La]^-$
Chemical Name	Di-aqua-bis-(L-ascorbate)-lanthanate(III) ion
UV-Visible (in nm)	254 (λ max in DMSO)
IR (in cm^{-1})	3382, 3364, 841 (H_2O), 3412, 3317, 3220 ($-OH$), 3030, 2917, 2907 ($-C-H$), 176 ($-C=O$), 1666 ($-C=C-$), 1442 ($-C-H$), 1146, 896 ($C-O$), 457 ($M-O$).
1H NMR	δ 4.72 – 4.86 ppm (m, H_4, H_5, H_6), δ 3.37 ppm (s, for $-CH_{asym}$), δ 4.6 ppm (s, for H_2O).
^{13}C NMR	δ 174.04 ($-C=O$), δ 156.22, 118.68 ($=C-OH$), δ 77.03, 69.76, 62.93, ($-CH-CH-CH_2$)
Mass	523 (M⁺)

Table -3: Spectrum analysis of Synthesised metal complex 3b

Molecular Formula	$[Ce(AA)_2(H_2O)_2]^-$
Chemical Formula	$[C_{12}H_{14}O_{12}Ce]^-$
Chemical Name	Di-aqua-bis-(L-ascorbate)-Cerate(III) ion
UV-Visible (in nm)	261 (λ max in DMSO)
IR (in cm^{-1})	3382, 3364, 841 (H_2O), 3412, 3317, 3220 ($-OH$), 3030, 2917, 2907 ($-C-H$), 176 ($-C=O$), 1666 ($-C=C-$) 1442 ($-C-H$), 1146, 896 ($C-O$), 457 ($M-O$).
1H NMR	δ 4.72 – 4.86 ppm (m, H_4, H_5, H_6), δ 3.37 ppm (s, for $-CH_{asym}$) δ 4.6 ppm (s, for H_2O).
^{13}C NMR	δ 174.04 ($-C=O$), δ 156.22, 118.68 ($=C-OH$), δ 77.03, 69.76, 62.93, ($-CH-CH-CH_2$)
Mass	524 (M⁺)

Table -4: Spectrum analysis of Synthesised metal complex 3c

Molecular Formula	[Nd(AA) ₂ (H ₂ O) ₂] ⁻
Chemical Formula	[C ₁₂ H ₁₄ O ₁₂ Nd] ⁻
Chemical Name	Di-aqua-bis-(L-ascorbate)-Neodymate (III) ion
UV-Visible (in nm)	254 (λ max in DMSO)
IR (in cm⁻¹)	3382, 3364, 841 (H ₂ O), 3412,3317,3220 (-OH),3030, 2917,2907 (-C-H), 176 (-C=O), 1666(-C=C-) 1442 (-C-H), 1146, 896 (C-O), 457 (M-O).
¹ HNMR	δ 4.72 – 4.86 ppm (m, H ₄ , H ₅ , H ₆), δ 3.37 ppm (s, for -CH _{asym}) δ 4.6 ppm (s, for H ₂ O).
¹³ CNMR	δ 174.04 (-C=O), δ 156.22, 118.68 (=C-OH), δ 77.03,69.76, 62.93,(-CH-CH-CH ₂)
Mass	528 (M⁺)

Table -5: Spectrum analysis of Synthesised metal complex 3d

Molecular Formula	[Gd(AA) ₂ (H ₂ O) ₂] ⁻
Chemical Formula	[C ₁₂ H ₁₄ O ₁₂ Gd] ⁻
Chemical Name	Di-aqua-bis-(L-ascorbate)-Gadolate (III)-ion
UV-Visible (in nm)	254 (λ max in DMSO)
IR (in cm⁻¹)	3382, 3364, 841(H ₂ O), 3412,3317,3220(-OH), 3030, 2917,2907(-C-H), 176 (-C=O), 1666(-C=C-),1442 (-C-H), 1146, 896 (C-O), 457 (M-O).
¹ HNMR	δ 4.72 – 4.86 ppm(m, H ₄ , H ₅ , H ₆), δ 3.37ppm(s, for -CH _{asym}), δ 4.6 ppm (s, for H ₂ O).
¹³ CNMR	δ 174.04 (-C=O), δ 156.22, 118.68 (=C-OH), δ 77.03,69.76, 62.93,(-CH-CH-CH ₂)
Mass	541 (M⁺)

Table -6: Anti-bacterial Activity of Synthesised Compounds

Compound code	<i>Salmonella typhi</i>		<i>Bucillus Subsniss</i>		<i>Staphylococcus aureus</i>		<i>Klebsiella pneumonia</i>	
	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm
3a	++	+++	++	+++	++	+++	++	+++
3b	++	+++	++	++	++	++	++	+++
3c	+	++	+	++	++	++	++	++
3d	+	+	++	++	+	++	+	++
SM	+++	++++	+++	++++	+++	++++	+++	++++

SM = streptomycin inhibition diameter in mm

Highly active = +++ (inhibition zones > 15)

moderately active = ++ (inhibition zone 10-15)

slightly active = + (inhibition 10)

inactive inhibition zone -6) for bacteria

Table 7: Antifungal activity of the synthesized compound derivatives

Compound code	<i>Aspergillus niger</i>		<i>Aspergillus flavus</i>		<i>Fusarium oxisporium</i>		<i>Trichoderma viride</i>	
	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm
3a	+	++	+	++	+	++	+	+++
3b	+	++	+	++	+	++	+	+++
3c	++	+++	++	+++	++	+++	++	+++
3d	++	+++	++	++	++	++	++	+++
GF	+++	++++	+++	+++	+++	++++	+++	++++

Std- Griseofulvin inhibition diameter in mm

Highly active = ++++ (inhibition zone > 20-25)

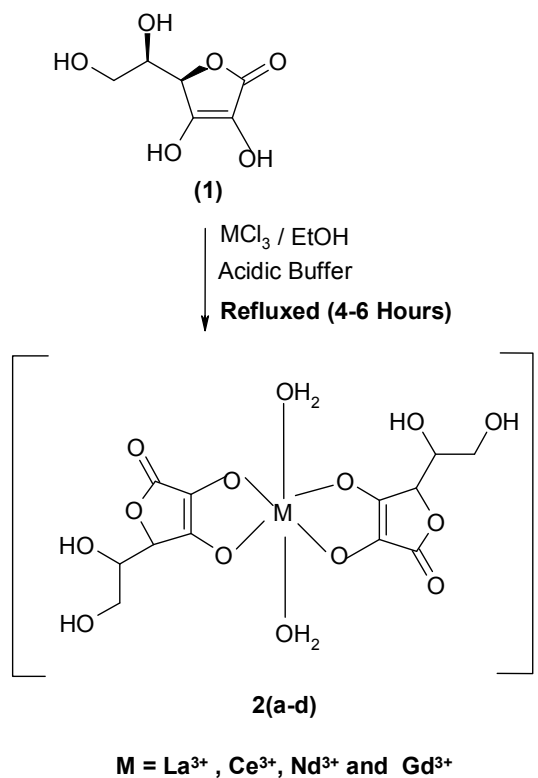
More Active = +++ (inhibition zone > 12-20)

Moderately active= ++ (inhibition zone 6-12)

slightly active = + (inhibition Zone less than 6)

Inactive inhibition zone - for Fungi

SCHEME 1



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